15 Grow 200 ml Buffered rich medium (Fermentation) with 1 ml of o/N grown cells

Both #1 & #3 were grown:

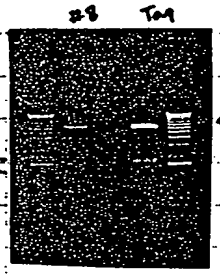
Grow at 28°C (the temp. of the incubator) until $A_{590} \sim 0.7$

Induce with IPTG (1mM) for 3 hrs → harvest.

Give cells to Adam Goldstein for purification/enzyme assay.

DH10B background

667Y Digest #8 with NgoA IV + XbaI in Read 1: } As before
Digest pTQ18-Taq with " " " " }



Purify both vector (Taq) plus the mutant fragment by gene clean as before:

Ligation: 15 µl DNA mix
4 µl 5X buffer
1 µl T4 ligase

Ligate 2 hrs / room temp

Transform DH5α mcr rec+ with 2 µl ligation.

Incubate plates at 30°C.

DH5α mcr rec+ background

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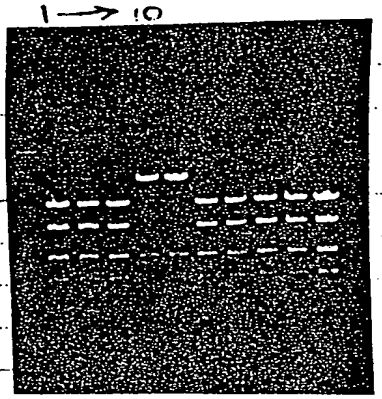
10% plating 154 colonies
90% plating >800

Inoculate 10 colonies for mini prep tommorrow. 2ml LB/amp. 30°C.

Standard miniprep from 1ml culture

DNA was dissolved in 150µl TE
Digest with Ase I (17µl DNA in NEB react 3)

1 → 10



Excpt for #4 & 5 all clones contains an extra Ase I site (the largest band was cut with an extra AseI site that created in the oligo)

Thus, pool all plasmids (except 4&5

Save cultures #1, 2 and #3.

Grow #1 and #2 for Adam — Purification.
~~200ml~~ 200 ml culture in Buffred rich medium.
Induced with 1mM IPTG / 5 hrs. at 30°C

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